

BULLETIN
OF
ENTOMOLOGICAL RESEARCH.

ISSUED BY THE IMPERIAL
BUREAU OF ENTOMOLOGY.

EDITOR : THE DIRECTOR.

VOL. XIII.

LONDON :
THE IMPERIAL BUREAU OF ENTOMOLOGY,
41, QUEEN'S GATE, S.W.7.
1922-23.

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CONTENTS.

ORIGINAL ARTICLES.

	PAGE
AUSTEN, MAJOR E. E.	
Further Notes on the Tabanidae of Palestine, with Descriptions of New Species	151
New and little-known Mesopotamian Blood-sucking Diptera (Families Simuliidae and Tabanidae)	275
A new East African Tsetse-fly (Genus <i>Glossina</i> , Wiedl.), which apparently disseminates Sleeping Sickness	311
BARRAUD, P. J.	
Some new Culicine Mosquitos found in India, and a Note on <i>Finlaya assamensis</i> (Theo.)	405
BRUNETTI, E.	
Two new Species of Tabanidae from Cuba	401
BRYANT, G. E.	
New Injurious Phytophaga from India and Brazil	261
BUXTON, P. A.	
On Fish and Mosquitos in Palestine	203
CARPENTER, DR. G. D. HALE.	
Report on a Test of a Method of attacking <i>Glossina</i> by Artificial Breeding-places	443
CHINA, W. E.	
A new Species of <i>Lygus</i> infesting Potatoes in Java (Rhynchota, Capsidae)	447
CLEARE, JR., L. D.	
Notes on the small Moth-borers of Sugar-cane in British Guiana ..	457
D'EMMERZ DE CHARMOY, D.	
An Attempt to introduce Scoliid Wasps from Madagascar to Mauritius	245
DRY, F. W.	
Notes on the Coconut Beetle (<i>Oryctes monoceros</i> , Ol.) in Kenya Colony	103
DUNN, L. H.	
Observations on the Oviposition of the House-fly, <i>Musca domestica</i> , L., in Panama	301
EDWARDS, F. W.	
• Mesquito Notes.—III.	75
On Some Malayan and Other Species of <i>Culicoides</i> , with a Note on the Genus <i>Lasiöhelea</i>	161
Four new African Mosquitos	397
FULLAWAY, D. T.	
A new Hymenopterous Parasite of the Australian Fern Weevil, <i>Syagrus fulvitaris</i> , Pasc.	201
GRANDI, PROF. G.	
Identification of some Fig Insects (Hymenoptera) from the British Museum (Natural History)	295
GREEN, E. E.	
On a small Collection of Coccidae from Mesopotamia, with Description of a new Species	469
JOHNSON, W. B., and LLOYD, DR. LL.	
First Report of the Tsetse-fly Investigation in the Northern Provinces of Nigeria	373

KUNHIKANNAN, K.	
An interesting Principle in Economic Entomology and some Useful Applications	404
LAMBORN, W. A.	
Some Problems of the Breeding-places of the Anophelines of Malaya :	
A Contribution towards their Solution	1
The Bionomics of some Malayan Anophelines	129
MACFIE, J. W. S.	
A Note on a Beetle which preys on Mosquito Larvae	403
MACFIE, J. W. S. & INGRAM, A.	
Certain Nurseries of Insect Life in West Africa	291
The early Stages of West African Mosquitos.—VI.	409
MACGREGOR, M. E. & GÉBERT, S.	
The Larva and Pupa of <i>Orthopodomyia arboricollis</i> , d'Emmerez de Charmoy, 1908	449
MARSHALL, DR. G. A. K.	
Some Injurious Neotropical Weevils (Curculionidae)	59
On the Australian Fern Weevils	169
MILLER, D.	
A Remarkable Mosquito, <i>Opifex fuscus</i> , Hutton	115
MORRIS, H. M.	
The Larval and Pupal Stages of the Bibionidae. Part II.	189
On a Method of Separating Insects and other Arthropods from Soil	197
PATTON, MAJOR W. S.	
Notes on the Calliphorinae. Part I. The Oriental Species	109
ROEBUCK, A.	
On the Occurrence of Leaf-eating Sawflies on Cereals in Britain	287
SPEYER, E. R.	
Mycetophilid Flies as Pests of the Cucumber Plant in Glass-houses	255
SWYNNERTON, C. F. M.	
The Entomological Aspects of an Outbreak of Sleeping Sickness near Mwanza, Tanganyika Territory	317
TILLYARD, DR. R. J.	
The Life-history of the Australian Moth-lacewing, <i>Ithone fusca</i> , Newman (Order Neuroptera—Planipennia)	205
TOTHILL, DR. J. D.	
Note Regarding Types of Some Tachinidae (Diptera) from India	181
UVAROV, B. P.	
Rice Grasshoppers of the Genus <i>Hieroglyphus</i> and their Nearest Allies	225
WATERSTON, DR. J.	
On the Chalcidoid Parasites of Psyllids (Hemiptera, Homoptera)	41
Two New Chalcidoid Parasites	183
A new Phytophagous Chalcid attacking Bamboo	307
On an Internal Parasite (Hym.—Chalcidoidea) of a Thrips from Trinidad	453
WILLIAMS, C. B.	
A Froghopper damaging Cacao in Panama	271
Mordvilko's Keys for the Determination of Aphids living continuously or temporarily on Gramineaceous Plants and Sedges	2:

MISCELLANEOUS.

Collections Received	127, 243, 371, 47
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ERRATA.

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- Page 70, line 18, for "*Zwalenburg*" read "*Zwaluwenburg*"
" 104, note, for "*rhinocetos*" read "*rhinoceros*"
" 161, line 28, for "Rec. Ind. Mus., iv" read "Rec. Ind. Mus., vi"
" 246, note, for "Bull. Ent. Research iii" read "Bull. Ent. Research viii"
" 301, note, 8th line, for "Manchester" read "Cambridge"

BULLETIN

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VOL. XIII.

1922.

SOME PROBLEMS OF THE BREEDING-PLACES OF THE ANOPHELINES OF MALAYA: A CONTRIBUTION TOWARDS THEIR SOLUTION.

By W. A. LAMBORN,

Malaria Bureau, Federated Malay States.

Knowledge of the breeding habits of the Anopheline mosquitos has advanced greatly since the days when it was considered that the larvae of any species were distributed indiscriminately in all and sundry collections of stagnant water. In the Federated Malay States entomological research, which has now extended over many years, tends to show that preferences by the various species for different kinds of breeding-places exist; and attempts have been made to define in relation to particular species, or groups of species, the types of breeding-place favoured.

Thus in 1904 Dr. G. F. Leicester,* who investigated the breeding habits of Anophelines at Klang, classified according to breeding habit those he found, as follows:—

- “(a) in mud-holes, *i.e.*, small holes made by the foot of a heavy animal or a waggon rut in a road: *Myzomyia rossii* [= *A. vagus*], *Cellia kochii*;
- (b) in stagnant shallow water supplied by rain and liable to dry up: *C. kochii*;
- (c) in swamp: *M. barbirostris*, *M. sinensis* [= *A. hyrcanus*], *M. umbrosus*, *C. kochii*;†
- (d) in marshy ground fed by a stream: *C. kochii*, *Nyssorhynchus nivipes* [= *A. fuliginosus*].”

In 1908 Dr. C. W. Daniels,‡ in a general paper on the breeding habits of Culicidae, described the larvae of *A. aitheni* (*treacheri*) and *A. maculatus* (*willmori*) as occurring in rapid streams; *A. rossi* as breeding in water much fouled with mud or fine silt; and *A. barbirostris*, *A. aconitus* (*albirostris*) and *A. kochi* as breeding in open country.

Dr. Malcolm Watson, discussing in 1911 the breeding-places of Anophelines, made numerous observations bearing on the distribution of Anopheline larvae.§ He pointed out (p. 116) that “*N. willmori* appears to prefer running water. There may be weeds but they must not too seriously interfere with the free current of water.

* “The Culicidae of Malaya,” Studies from the Institute of Medical Research, Kuala Lumpur, iii, pt. 3, p. 10, 1908.

† Throughout this paper the new nomenclature for certain species has been followed, the species formerly termed *A. rossi* var. *indefinitus* being called *A. vagus*; the Malayan form of *A. rossi*, Giles, becomes *A. subpictus* var. *malayensis*; and *A. sinensis* becomes *A. hyrcanus*.

‡ “The Breeding Habits of Culicidae,” Studies from the Institute of Medical Research, Kuala Lumpur, iii, pt. 3, p. 3, 1908.

§ The Prevention of Malaria in the F.M.S., 1911.

M. rossii is at the opposite end of the scale from *N. willmori* and is a puddle breeder." Elsewhere (p. 111) in the same book he describes the situations in which he obtained Anopheline larvae in the Bukit Gantang valley—enormous numbers of *A. barbirostris* and *A. hyrcanus* (*sinensis*) in the paddy fields, *A. subpictus* var. *malayensis* (*rossii*) in pools near to the houses and *A. aconitus* (*albistrostris*) in a stream running through paddy fields and in swampy grass through which water was flowing slowly.

Mr. C. Strickland* endeavoured, after a five years' study of the question, to define what is a typical breeding-place of a certain species by tabulating certain characteristics of the surroundings in which it was found; but as Dr. Hacker has pointed out,† "the attempt showed the impossibility of doing this, owing to the very large number of not accurately measurable characteristics that have to be classified." Dr. Hacker nevertheless continued the same line of investigation in the hope of establishing a definite association between certain species and a definitely recognisable type of breeding-place, and basing his conclusions on an examination in the course of three years of no fewer than 4,949 breeding-places and the identification of 39,605 specimens, suggested‡ that the species may be grouped in the following manner:—

"Group 1, depending on a common preference for small open pools or open hilly country: *A. kochi*, *A. vagus*, *A. maculatus*, *A. karwari*, and *A. ludlowi*. The last of these species may separate itself from this group as the chief member of a salt-water fauna when further data have been collected.

"Group 2, depending on a preference for large swampy pools or low-lying country: *A. barbirostris*, *A. hyrcanus*, *A. aconitus*, *A. fuliginosus*, *A. subpictus* var. *malayensis*, *A. separatus*, *A. tessellatus*, *A. umbrosus*, and probably *A. aurorostris*. The last three may separate themselves later into a jungle swamp fauna, leaving the rest of the group as an open swamp fauna.

"Group 3, depending on a preference for jungle, probably hilly jungle: *A. aitkeni*, *A. leucosphyrus*, *A. albolaeniatulus* var. *montanus* and *A. novumbrus*.

"Group 4, species with highly specialised breeding-places: *A. asiaticus*."

He, however, weakens the validity of his conclusions by himself pointing out (p. 24) "that the groups are not sharply delimited shows that the species are not strictly limited in their choice of breeding-place but have a certain amount of adaptability." Elsewhere (p. 7) Dr. Hacker states that he, also, has found it "quite impossible to classify the characteristics of breeding-places so as to constitute a description of the typical breeding-place of each species." The latitude as to breeding habits is indeed brought out in his own "Association Value Tables" put forward in the same paper, e.g. (p. 14) he finds in 544 collections an association of *A. kochi* (group 1) with *A. hyrcanus* 78 times and with *A. barbirostris* 30 times (these two species being placed by him in group 2): an association in 816 collections of *A. vagus* (group 1) with *A. hyrcanus* 84 times and with *A. barbirostris* 64 times.

Although the experience gained from the work of one year hardly entitles the writer to express an authoritative opinion, it may be said summarily that the result of his own investigations has been to enforce Dr. Hacker's qualification at the expense of his main thesis. In the face of the mass of data accumulated and presented below it is difficult to accept as of any great value the suggestion that the open country Anophelines (which are all comprised within groups 1 and 2) prefer for breeding either "open hilly country" or "low-lying country"; for the larvae of all species at all common were obtained at the same level and in great abundance round Kuala Lumpur, and though some of the species certainly seem to exhibit preference either for "small open pools" or for "large swampy pools," exceptions are quite numerous.

For the purpose of the present paper some actual examples of the exceptions which came within the experience of the writer may be recorded. *A. vagus* comes

* Unpublished report at Malaria Bureau.

† F.M.S. Malaria Bureau Annual Report for 1918.

‡ F.M.S. Malaria Bureau Reports, ii, 1920.

within the group of Anophelines which are said to have a preference for breeding in small open pools or open hilly country. But a collection of larvae from the bottom of an old boat, which contained a mixture of rain-water and water that had leaked into it from a fishpond on which it floated, afforded 162 larvae of this species in pure culture; and a collection, made at the same time from the fishpond itself, yielded 35 larvae of *A. vagus* also, 132 larvae of *A. hyrcanus*, 36 of *A. barbirostris*, 6 of *A. fuliginosus* and one of *A. aconitus*.

A collection on 1st December 1920 from small muddy pools afforded 368 larvae of *A. vagus* with 2 of *A. kochi*, 62 of *A. hyrcanus* and 28 of *A. barbirostris*, the two latter species being usually found in large swampy pools or low-lying country. In another collection, made on 15th April 1920 from small muddy pools in the same locality, 30 larvae of *A. barbirostris*, 4 of *A. hyrcanus* and 3 only of *A. vagus* were obtained. An answer to the speculation as to whether in a period of drought *A. vagus* ceased to breed, owing to an absence of its favoured breeding-places, small muddy pools, or availed itself of other waters, was provided by a collection made at such a season, in a fishpond. Here were secured, on 29th March 1920, 244 larvae of *A. vagus* with 53 of *A. hyrcanus* and 3 of *A. barbirostris*, whereas in seven collections made from the same pond up to 22nd of the month, a wet period, there were only 72 larvae of *A. vagus* in a total of 1,697 other larvae. A further collection from the fishpond, on 26th August 1920, afforded no fewer than 628 larvae of *A. vagus*, 2 of *A. subpictus*, 5 of *A. fuliginosus*, 18 of *A. barbirostris* and 105 of *A. sinensis*.

It is evident, then, that no conclusions sufficiently valid to be of any practical value can be drawn as to the species of larvae to be found in a breeding-place from characteristics of its environment. Notwithstanding this, the object of the present paper is to advance the argument that Anophelines are not merely selective "to some extent," but are selective to a high degree, and that this selective tendency depends on other factors than the mere environment of a breeding-place. It will be shown in the case of *A. aconitus*, to which study was largely restricted, that choice has been constant over a very long period of time, there being other comparable situations available in which none of these larvae were found. An attempt will be made to consider some of the factors on which such choice may well depend.

* *A. aconitus* is one of the dominant Anophelines of the Malay States. In Kuala Lumpur it is certainly the most abundant of all, a total of no less than 5,753 adult specimens out of a grand total of 11,001 of these mosquitos having been obtained in about nine months by the writer in the course of some work having as its object the determination of the seasonal prevalence, if any, of mosquitos. It is an open-country breeder and, as has been pointed out by Dr. Hacker in the report referred to, has a preference for large reedy ponds and open deep swamps, being there associated with *A. barbirostris*, *A. hyrcanus*, *A. fuliginosus*, *A. subpictus* var. *malayensis* and some other species.

For the purpose of estimating the constancy of this Anopheline in her selection of a breeding-place it was arranged that collections, to include all Anopheline larvae, should be made at a particular place, a large open reedy swamp in low-lying ground on the outskirts of the town and subject to periodic inundation by the overflow from various streams. During a period of drought it became dry over a large area, but water was constantly found in the small open ponds which still remained. In this swamp preliminary examinations had shown the presence of a large number of these particular larvae. Collections were made here at irregular intervals during October up to the 20th. Thereafter they were made twice weekly by the same number of collectors, seven; working on each occasion for the same period of time, about three hours.

Until the absolute certainty of their determination by microscopic examination had been realised, the larvae in each collection were determined by breeding out. When, after an examination of hundreds, no single error had been found (for species difficult to determine by larval examination did not come into consideration) this

unnecessary labour was dispensed with and sole reliance was placed on the use of the microscope for identification purposes. The larvae in each collection were determined shortly after their arrival in the laboratory, and the results are given in full in the following Table I.

TABLE I.

Date.	<i>A. aconitus.</i>	<i>A. barbivostris.</i>	<i>A. hyrcanus.</i>	<i>A. fuliginosus.</i>	<i>A. vagus.</i>	<i>A. hochi.</i>
6.x.20	714	5	1	1	—	1
9.x.20	210	6	—	—	—	2
11.x.20	428	—	—	—	—	—
12.x.20	259	—	2	—	—	—
15.x.20	569	—	—	—	—	—
19.x.20	477	—	—	—	—	—
20.x.20	427	—	12	—	—	—
23.x.20*	236	—	—	—	—	—
25.x.20	337	—	3	—	—	—
26.x.20	770	3	7	—	—	—
27.x.20	218	—	—	—	—	1
30.x.20	24	—	—	—	—	1
4.xi.20	128	—	—	—	—	—
12.xi.20	135	—	—	—	—	—
15.xi.20	408	45	21	6	—	—
18.xi.20	381	23	24	11	—	1
25.xi.20	84	8	15	—	1	8
29.xi.20	49	17	56	2	1	5
2.xii.20	14	25	137	—	—	11
6.xii.20	48	106	340	4	—	4
9.xii.20	198	61	109	2	1	—
13.xii.20	468	132	155	12	—	—
16.xii.20	181	91	114	4	—	—
20.xii.20	572	62	170	2	—	—
22.xii.20	355	105	90	9	—	1
28.xii.20	55	192	80	19	—	—
30.xii.20	61	204	136	29	—	—
3.i.21	405	256	137	21	—	—
7.i.21	262	137	62	23	—	—
10.i.21	392	167	152	25	—	—
13.i.21	286	91	69	14	—	—
17.i.21	150	160	30	5	—	—
21.i.21	134	169	29	3	—	—
24.i.21	215	75	131	9	—	2
27.i.21	268	123	72	7	—	—
31.i.21	200	236	51	11	—	—
3.ii.21	141	75	95	19	—	—
7.ii.21	78	44	23	5	—	—
14.ii.21	128	98	28	16	—	—
17.ii.21	101	194	60	15	—	—
21.ii.21	68	250	28	6	—	—
24.ii.21	74	29	10	13	—	—
28.ii.21	126	248	38	58	—	—
3.iii.21	72	135	46	46	—	—
7.iii.21	120	132	37	41	—	—
10.iii.21	200	160	37	4	—	—
14.iii.21	120	160	97	12	—	—
17.iii.21	72	72	29	7	—	—
21.iii.21	20	213	20	17	—	—
24.iii.21	10	8	3	11	1	—
29.iii.21	105	191	12	12	—	—
31.iii.21	80	150	70	3	—	—
4.iv.21	62	114	57	3	—	—
7.iv.21	200	110	100	25	—	—
11.iv.21	53	67	123	27	—	—
Totals	11,938	4,949	3,118	559	4	37

* *A. tessellatus*, 1.

During the period from October to April, then, the larvae of *A. aconitus* were found constantly and in such abundance that they almost outnumbered the larvae of the seven other species of Anophelines taken at the same time.

The overwhelming dominance of this species over all others up till the end of October, and thereafter the sudden relative increase in the others, is incidentally to be noticed.

For the purpose of comparison, larvae were collected under similar conditions regularly twice a week from 1st November from another breeding-place, distant about a mile in direct line from the former one, where preliminary investigations had shown the absence of the larvae of *A. aconitus*, though other members of Dr. Hacker's Group 2 were excessively abundant. This was a very large artificial pond about 50 yards square and kept free from reeds and coarse vegetation. It seemed to be of more or less uniform depth, which averaged four to five feet, and it was used by the Chinese owners for the cultivation of fish. The data obtained by these collections are given in the following table:—

TABLE II.

Date.	<i>A. aconitus.</i>	<i>A. barbirostris.</i>	<i>A. hyrcanus.</i>	<i>A. fuliginosus.</i>	<i>A. subpictus.</i>	<i>A. vagus.</i>
29.x.20	—	57	26	5	—	3
1.xi.20	—	36	17	7	1	23
19.xi.20	—	24	48	6	—	19
20.xi.20	—	36	132	6	—	—
25.xi.20	—	51	201	4	1	3
26.xi.20	—	82	97	—	1	—
30.xi.20	—	94	116	3	—	—
3.xii.20	—	62	133	—	—	5
7.xii.20	—	30	150	2	—	2
10.xii.20	—	42	157	3	—	18
14.xii.20	—	103	208	13	—	22
17.xii.20	—	98	108	4	—	10
21.xii.20	—	242	175	1	1	11
24.xii.20	—	52	92	—	9	4
28.xii.20	—	93	76	4	9	57
31.xii.20	—	155	170	—	—	16
4.i.21	—	97	51	—	—	—
7.i.21	—	70	87	—	—	5
11.i.21	—	114	47	—	—	—
14.i.21	—	100	73	—	—	2
20.i.21	—	44	39	—	—	36
21.i.21	—	76	40	—	—	4
25.i.21	2	25	167	2	4	110
28.i.21	—	110	80	—	—	7
1.ii.21	—	55	263	7	—	40
4.ii.21	—	74	230	—	—	22
7.ii.21	—	54	100	—	—	15
11.ii.21	—	11	88	—	—	2
15.ii.21	—	51	332	3	—	11
18.ii.21	—	48	240	1	—	13
22.ii.21	—	60	371	—	—	13
25.ii.21	—	15	289	1	—	36
1.iii.21	—	34	259	—	1	36
4.iii.21	—	16	152	—	—	2
8.iii.21	—	13	401	2	—	26
11.iii.21	—	17	229	—	—	4
15.iii.21	—	11	304	1	—	1
18.iii.21	—	4	113	3	—	—
22.iii.21	—	13	115	9	—	3
29.iii.21	—	3	53	—	2	244
1.iv.21	—	80	200	—	—	9
5.iv.21	—	18	177	1	4	130
8.iv.21	—	20	247	3	—	120
Totals	2	2,589	6,743	91	31	1,093

The entire absence of *A. aconitus*, except for two individuals, in a collection numbering 10,549 and comprising five other species, as shown in Table II, offers a most striking contrast to its relative proportions in the parallel collections, as shown in Table I, in which out of a total of 20,605 larvae, including six other species, no fewer than 11,938 were those of this Anopheline. To what causes can its absence be due? To what is due the overwhelming abundance of this species elsewhere? If due to differences in the nature of the breeding-places, in what do these consist?

The mere presence of reeds, affording a measure of shade, would hardly seem to account for it, as the larvae were obtainable elsewhere in situations entirely free from such growth and exposed to the overhead sun. The difference in size of the ponds and the depth of the water has also no bearing on the matter: one knows of other breeding-places equal in point of size and depth of water, in some of which the larvae of *A. aconitus* are found though absent in others.

For further study of the question of selection of breeding-place, a little group of four other small ponds and one little reedy swamp on the outskirts of another quarter of the town were selected in March 1921. The relative position of these is shown on the accompanying map (fig. 1).

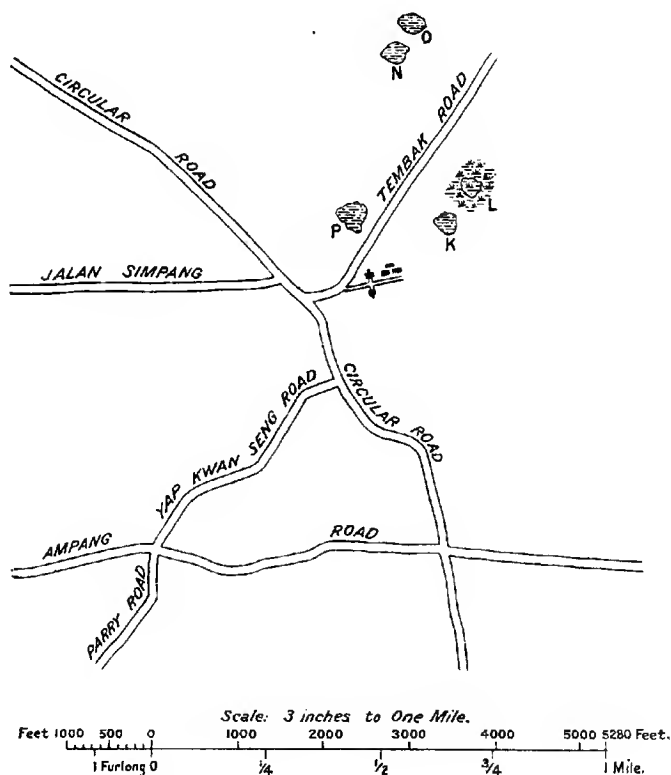


Fig. 1. Sketch-map to show the relative positions of the ponds examined for *Anopheles* larvae.

The swamp (L) is a natural feature: the ponds (K, N, O, P) were probably old mining holes, and were approximately of the same size and depth.

The swamp is a shallow one, overgrown with reeds and coarse grass, but open here and there with large stretches probably of deep water. At the time collections were made a slow current of water was moving. The larvae here obtained by two collections are shown in the following table :—

Date.			<i>A. aconitus.</i>		<i>A. barbirostris.</i>		<i>A. hyrcanus.</i>
23.iii.21	98	..	47	..	18
31.iii.21	65	..	8	..	17

The pond (K) was a collection of stagnant water situated among young *Hevea* trees, but unshaded from the overhead sun. A few tufts of coarse vegetation grew in one corner but, apart from that, it was almost free from floating aquatic plants.

The larvae obtained by two collections are shown in the following tables :—

Date.			<i>A. aconitus.</i>		<i>A. barbirostris.</i>		<i>A. hyrcanus.</i>
22.iii.21	52	..	9	..	9
31.iii.21	8	..	37	..	—

Thus in breeding-places (K) and (L), close together, but showing very different characteristics, *A. aconitus* was by far the most dominant species in three collections, though in the fourth, that obtained on 31st March, there was a marked diminution in the numbers obtained.

Ponds (N) and (O), situated about 100 yards away from pond (K) and swamp (L), were characterised by a dense growth of large surface aquatic plants, with rushes and some reeds here and there at the margins. On pond (N) the surface plants were chiefly the "Japanese Hyacinth" and "Cabbage weed" and on pond (O) the latter only. These plants were probably more or less cultivated by a Chinese farmer, for they are used for the purpose of feeding pigs.

The following larva collection was obtained from pond (N) :—

Date.			<i>A. aconitus.</i>		<i>A. barbirostris.</i>		<i>A. hyrcanus.</i>
5.iv.21	—	..	197	..	42

In pond (O) the following larvae were obtained :—

Date.			<i>A. aconitus.</i>		<i>A. barbirostris.</i>		<i>A. hyrcanus.</i>		<i>A. fuliginosus.</i>
5.iv.21	—	..	—	..	90	..	40

The remaining breeding-place examined was a pond of stagnant water (P) more shaded by trees than the four other places and situated within 50 yards of a Chinese house and about 250 yards from the other breeding-places. Tufts of grass dotted the surface thinly, and there was no surface vegetation. Neither was there any vegetation on the banks. A collection made here afforded the following larvae :—

Date.			<i>A. aconitus.</i>		<i>A. barbirostris.</i>		<i>A. sinensis.</i>		<i>A. fuliginosus.</i>
5.iv.21	—	..	6	..	8	..	76

There is difficulty, therefore, in believing that any outward characteristics of a breeding-place influence the preference of *A. aconitus*. To what causes, apart from these, can the differences in distribution be due? In view of the abundance of Anophelines, their fertility and, so far as is known, the comparative freedom of their larvae from attack by natural enemies, it might be thought that oviposition is entirely haphazard, the female trusting to lucky chance, like some of the Bombyliid flies for

instance, that a few of her ova, more fortunately placed than the majority, will ultimately afford imagos to carry on her race, the larvae which develop from ova less fortunately dropped dying out. For example, an *A. maculatus* may be presumed to have oviposited without having exercised any great selection in a fish-pond, a small shallow muddy pool, and a deep sheltered grassy drain. The latter situation is one in which the larvae are commonly found, and their occurrence may be a measure of the suitability of the place, the larvae developing from ova deposited on the other waters perishing at an early stage. But judging by what one sees in the case of other insects, the presence of *A. aconitus* in the situations referred to is more likely to be due to a very judicious selection by the female parents in the best interests of their offspring.

It is a far more general rule in the insect world that the female parent deposits her ova with almost unfailing precision in the situations best suited for the welfare of the larvae. For instance, the female butterfly, of species having leaf-eating larvae, lays her eggs almost invariably on the softest and most succulent part of the food-plant, often selected after a search lasting hours, and probably days, so giving her offspring the best possible start in life; she will often die in captivity rather than oviposit on an unsuitable plant. Some of the myrmecophilous LYCAENIDAE oviposit in the very track of ants, so ensuring the due carriage of the egg to the ants' nest; and, as the writer showed in the case of a species having larvae with predacious habits, the butterfly is at pains to oviposit actually on the particular little "bug" on which its larva feeds. The female tsetse-fly exercises such meticulous discrimination in regard to the choice of a spot in the earth in which to deposit her maggot that, though the fly swarms in certain districts of Africa, its breeding-places were discovered only after long and patient search by skilled observers. The bot-fly deposits its ovum more often than not on a part of the coat where it can most readily be reached by the tongue of its equine host.

Some definite evidence of selection by *Culex fatigans* as to its breeding-places was obtained by examination at Kuala Lumpur of a number of pools—about fifteen—made by the passage of carts and transport animals through soft clayey soil at the side of the road. On only three were the egg rafts of the *Culex* found, and then in numbers on each—four on one pool, three on a second and seven on a third. All the pools were under the same conditions in regard to exposure; all were absolutely unshaded; there was no grass round any; there appeared to be an equal absence in all of small vegetation, and there was no great variation as regards depth.

Some experimental evidence bearing on the question was obtained in the laboratory in the case of this *Culex*. In three large vessels water, in which on 13th August rice had been boiled, was left standing. The water soon became fetid and a white downy fungus growth developed. On the surface of the water in one of the jars a *Culex* raft was discovered on 20th August: a second was found on 22nd and a third on 26th, none at all being deposited on the control vessels, side by side with the first. The dissociation of the eggshells comprising a raft may take a considerable time after the hatching of the larvae, so that a raft may long serve to attract an ovipositing female.

Laboratory experience with *Stegomyia albopicta* was precisely the same. This mosquito was an absolute nuisance by reason of its ovipositing in vessels containing the larvae of various Anophelines, so necessitating the constant removal, for the sake of the Anophelines, of their stronger and more active competitors. The ova were found day by day in the same particular bowls, though others containing similar Anopheline larvae in water from the same place were available. The careful selection made even by this mosquito was further exemplified by the discovery in the laboratory, on 17th August, of 46, and on 30th August, of 72 ova, on the water of the same bowl, the only one in which *Stegomyia* was being reared out of twenty-five all full of water, and kept under the same conditions as regards temperature and light.

It is therefore only in accordance with expectation that the female Anopheline, also, should exercise very careful selection of a breeding-place. Were there not masses

of data available, such as have been quoted as to *A. aconitus*, there is strong presumptive evidence for careful selection on the part of *A. maculatus* that, when hard pushed for favoured breeding-places, it does not seem to avail itself of other collections of water. The water which formerly stagnated in the open ravines round Federal Hill and Carcosa in Kuala Lumpur at one time swarmed with larvae of *A. maculatus*, which have now almost been eradicated through the application of engineering science (subsoil pipes, open concrete drains, etc., most judiciously laid) to entomological observation. But though the species must here be very hard pushed for breeding-places, it would seem still to show its marked preference for such a type of breeding-place by depositing its ova at any point in the drains where any little temporary deficiency of the work, even a mere fissure in the cement, affords water sufficiently still. The Anopheline does not seem to select other possible breeding-places. Though a large sheet of open water is available, the writer found on one occasion only two or three of these larvae in it and, though under his direction an examination was made of numerous possible breeding-places in the neighbourhood, which afforded a vast number of *Stegomyia*, in no single instance was *maculatus* obtained.

It is, therefore, at least probable that the presence of larvae of *A. fuliginosus*, found in large numbers in almost pure culture in a pond near Kuala Lumpur, was due to careful parental selection, 149 being obtained on one occasion with one of *A. hyrcanus*; and 100 more, six days later, at the same place.

Dr. Malcolm Watson related to the writer how, many years ago, when a road was cut through a hill at Klang, he made search for Anopheline larvae in the springs exposed. In one only did he find them, and in this a cow had deposited manure. The larvae were those of *A. vagus* and they were of all sizes. This experience was paralleled by one of the writer's, for in the course of a search for larvae of this species, in a number of cart ruts and hoof prints, in only three out of about a dozen were they found. The larvae were those of *A. vagus* in considerable numbers and of various sizes, so, most probably (though not necessarily, since the size of a larva is not always a guide to its age) the progeny of several females.

Assuming that there is refined selection by the ovipositing Anopheline, what are the influences likely to guide her in her choice? One supposition was that the presence of already existing larvae might attract the female.

To the naturalist, acquainted with instances of far more delicate perceptions on the part of insects than would be called for in the detection by an Anopheline of the larvae of its own species, there is no difficulty at all in supposing that a female *A. vagus*, for instance, having discovered the few small pools from which were taken on 18th August no less than 250 larvae of her own species, 95 others too small to be classified with certainty and 49 pupae probably of this species, might well be influenced in her decision to oviposit by the evidence of conditions so auspicious to the offspring of others; and that a pregnant *A. maculatus* might be similarly influenced on arriving at a grassy drain from the water of which were readily collected, on 21st July, 283 of these larvae, on 30th July 222, on 28th July 183, and on 4th August 126, with a considerable number of pupae on each occasion.

It seemed as if the point might readily be settled by affording captive mosquitos the alternative opportunity of ovipositing on plain tap water, and tap water containing larvae corresponding to the species experimented with. This was attempted, two bowls of plain water and one containing the larvae being supplied, in a long series of experiments, to ovipositing females of various species. No predilections by the female parents were discovered for the bowls containing the larvae, the greater number of the batches of ova being deposited on the plain water.

Had positive results been obtained, they would have afforded useful data, but it was impossible to make such an experiment under perfectly natural conditions. The mosquitos, though carefully fed and screened from an undue amount of light during the day, bore captivity hardly, injuring themselves in their almost constant endeavour

to escape. It is therefore probable that all other instincts were subordinated to those of self-preservation, and that oviposition was effected only when it could be no longer deferred, and then in the first bowl on which the insect happened to chance.

However, the female mosquito, like other insects, is probably influenced in her choice primarily by the odours characteristic of particular spots, which connote the general suitability of the situation as to temperature, composition of water, presence of particular foods. The guiding odours may or may not be such as are perceptible to the imperfect sense of smell possessed by the ordinary human being. For instance, *A. umbrosus*, breeding largely in jungle pools (when away from the coast), may conceivably be attracted thereto by the moist rich smell of the rotting leaves necessarily associated with the presence of shade and of water, conditions which may afford these larvae suitability of temperature and may favour the growth of the foods on which they depend. The odour of many breeding-places which harbour the larvae of *A. vagus* in the neighbourhood of native habitations is often such as grievously offends the nostrils and may well serve to attract the mosquitos from afar to oviposit. The water in which large collection of *A. aconitus* was obtained seemed to be almost odourless to human nostrils.

If odour influences the female it should be possible to mask it experimentally by adding to the water of a breeding-place a strongly odorous substance, the expectation then being that the female, repelled by it, or unable to detect the guiding odour, would no longer oviposit. The two bodies with pungent odours which first came to mind were chlorine and formalin; and a test was made with the latter. The breeding-place selected was a pool in secondary jungle of considerable size, long known to be favoured as a breeding-place by *A. umbrosus*. This had the advantage that it was of the size suitable for such an experiment; it was in a shaded situation, in which, owing to the improbability of rapid evaporation, the formalin was likely to be effective over a longer period than in a pool out in the open; and the data in reference to the collection of larvae from it extended over a considerable period of time, showing that it was an old-established breeding-place. Formalin was added, until a very faint odour was perceptible and this was detected again a few hours later. Thereafter collections of larvae of *A. umbrosus* were made after four or five days. The addition of so small an amount of formalin would probably have little effect on larvae already approximating maturity but, besides preventing oviposition, might well also prevent hatching, or might have a prejudicial effect on young and delicate larvae. But no results at all were obtained, for the larvae were as numerous after the experiment as before it.

No ova of *A. umbrosus* were available for experimental purposes, but about half of a batch of 166 freshly laid ova of *A. vagus*, placed on a solution of formalin just strong enough to afford a perceptible odour, duly hatched out equally well as the control half, and the young larvae seemed unaffected for three days, moving their mouth brushes as if hoping to obtain food. They then gradually died off. An attempt to obtain positive evidence of the predilections of the female Anopheline as to waters was made by supplying the captive insects with two alternative waters in addition to some from the breeding-place in which the larvae were readily obtainable in the expectation that they would select the latter. The water samples were placed in small bowls side by side in lamp glasses containing the insects. The results, again, were entirely negative; no preferences were shown. But here again the experiments are open to the objection that the insects were bent mainly on self-preservation. Furthermore the odours from the different waters may well have blended, so misleading the insects, or may have been lost, the gases in solution coming off rapidly in the tropics.

The question as to how far the larvae of different species can thrive in waters other than those in which they are usually found seemed a more promising line of investigation and was initiated by a series of experiments made with a view to determining if the hatching of the ova of the various species is, to any degree, prejudiced by their transfer to water in which they are not usually found. The results of the experiments were again negative where natural waters were concerned.

The newly laid ova of *A. aconitus*, for instance, which were tested on tap water and on the water from small muddy pools on unfrequented roads, from the fishpond referred to, from the cesspit of a Chinese house, and from a jungle pool, all hatched. The ova of *A. aconitus* and of *A. vagus* duly hatched when transferred within a few hours of oviposition to water which had been thoroughly boiled a few hours previously.

An experiment of some interest consisted in the transfer of 67 newly laid ova of *A. vagus* to water in which rice had been boiled a few days previously and which was therefore acid and malodorous, the rice fragments forming a culture medium for a species of mould. No hatching at all took place, though of 25 ova of the same batch tested as a control on fresh water every single one hatched. Though this medium was so unpropitious to this species of Anopheline it turned out to be singularly favourable to a species of *Culex*, three rafts of ova deposited thereon affording hundreds of larvae which fed up first on the moulds and then on the rice fragments, so that the water became clear, and being placed in a light situation developed, at the end of about a month, a strong growth of green algae. These were allowed to thrive undisturbed for another month, when more ova of *A. vagus* were transferred to it. These now hatched and the larvae attained about half size, then succumbing. A sufficiency of nutritive matter for the development of algae may perhaps explain the very occasional presence of Anopheline larvae in artificial breeding-places. In another similar experiment about 80 ova of *A. vagus* were placed on the medium. All failed to hatch, though 10 ova, as a control on tap water, duly afforded larvae. A succession of larvae of *Stegomyia albopicta*, hatching from eggs deposited by stray females on the material, which was in an open glass jar exposed in a well-lighted situation, bred up, though during such time as the medium remained acid and foul, newly hatched larvae of *A. vagus* transferred to it soon perished.

By way of further experiment 20 ova of *A. vagus* and an equal number of those of *S. albopicta*, all newly laid, were placed on the surface of a thin mucilage of rice boiled up two days previously. Most of the eggs of the *Stegomyia* hatched, but the majority of the larvae died, though one managed to survive until the tenth day. Not a single larva of *A. vagus* hatched, although control ova duly afforded larvae.

The ova, then, would hatch on all normal media. The growth and development of larvae in water from breeding-places other than those in which the species experimented with is usually found was also made the subject of enquiry. The results again were not in accordance with expectation: the larvae of the various open-country species could be bred in the laboratory to maturity in natural media in which numerous examinations had shown constantly the entire absence of the larvae of the species under experiment. For instance, almost within a stone's throw of the swamp referred to (Table I) in which the larvae of *A. aconitus*, *A. hyrcanus*, *A. fuliginosus* and *A. barbirostris* were found in such great abundance, there was a large pond of stagnant water, well shaded by rubber trees, varying in size according to season, but during most of the year, about ten yards wide and twenty long, with a depth of one to three feet. The water, surface drainage, partly from a road and partly from a plantation in which there was a small Chinese and Malay settlement, was always turbid and sometimes malodorous both from vegetable and animal pollution, and a green scum, due to the presence in almost pure culture of Protozoa (determined by Dr. Stanton as a species of *Euglena*, probably *viridis*) was present on the surface during the period the investigation was carried out. The only Anopheline larvae ever found in this water, in spite of their proximity in such great abundance in the water of swamp (A) near by, were those of *A. vagus*, and these but sparingly. In view of the scantiness of these particular larvae, suggesting rather unfavourable environment (since they abounded in road pools near by) and of the entire absence of other species, suggesting complete unsuitability of conditions for them, any success in breeding the open-country Anophelines in this water was hardly to be anticipated. But expectations were falsified; the water proved to be the best medium of many tried by the writer

for the purpose, and it was used for rearing for the study of variation and other purposes large families of these mosquitos from known female parents. The results will be presented in another paper; for the purpose of the present one it will suffice to give a few of the data obtained. These are given in the following Table III.

TABLE III.

Species.	Number of ova.	Number of adults obtained.		
		Males.	Females.	Totals.
<i>A. aconitus</i>	176	47	35	82
	91	30	31	61
<i>A. barbirostris</i>	98	30	14	44
	115	39	30	69
<i>A. hyrcanus</i>	75	42	31	73
	117	41	40	81
<i>A. maculatus</i>	30	9	17	26
	68	17	15	32
<i>A. karwari</i>	97	20	27	47
<i>A. subpictus</i> var. <i>malayensis</i>	88	37	20	57
	97	32	29	61
<i>A. vagus</i>	146	42	33	75
	72	23	23	46
<i>A. ludlowi</i>	120	14	22	36
	50	14	6	20
<i>A. fuliginosus</i>	46	22	18	40
	75	16	18	24
<i>A. kochi</i>	110	31	53	84
	82	20	18	38
<i>A. tessellatus</i>	38	10	3	13

The surprising fact arises, then, that all the common Anophelines could be bred from the egg in a medium in which none except *A. vagus* existed in nature. A still more surprising result was that any success at all was met with in the case of *A. ludlowi*, a species limited in the Federated Malay States (though not in Java) to the coastal area, by reason of its preference for brackish pools as breeding-places. The case is the more interesting by reason of the observation made by Dr. Hacker, that when the salinity of breeding-places favoured by this species becomes diminished owing to rainfall the larvae are no longer found, but may be replaced by those of *A. vagus*.* All the results were not uniform, however. It was found, especially with *A. aconitus*, that unless the water was changed daily, the larvae soon died out (neglect to change it on a Sunday caused the death of several broods), and that other larvae, especially *A. maculatus* and *A. vagus*, though less prejudicially affected, often maintained themselves in such water unchanged for several days. On one occasion a number of larvae of *A. aconitus*, comprising two families and numbering 108, had thrived to such a degree in this *Euglena*-containing water that their pupation was expected within a day or two. On transfer of the larvae one morning to fresh bowls of water it was noticed that fetor was arising from it and, as it had already been appreciated that these larvae are more delicate than others, some misgiving was felt as to them. These fears were soon realised; the larvae ceased to feed continuously, the mouth-brushes working intermittently only, and within 36 hours the whole of both families had perished, the larvae of the other species changed into similar water being apparently uninfluenced. It was to be expected that the larvae of this species would succumb in any foul water. To test this, about half of a batch of 59 ova were placed on 9th

* F.M.S. Malaria Bureau, Reports, ii. p. 38.